Effects of 4-hydroxyandrostenedione and exogenous testosterone on blood concentrations of oestradiol and oviducal embryo transport in the rat

M. L. Forcelledo and H. B. Croxatto
Laboratorio de Endocrinología, Departamento de Ciencias Fisiológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile

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ABSTRACT

The effect of decreasing oestrogen secretion on the oviducal migration of embryos was investigated in pregnant rats. The reduction of oestradiol production was achieved by administration of the aromatase inhibitor 4-hydroxy-4-androstene-3,17-dione (4-OH-A) at various times after coitus.

When 4-OH-A was administered from days 2 to 5, nearly half the embryos were retained in the oviducts at midday on day 5 of pregnancy, in contrast with control animals in which all embryos were transferred to the uterus. Shorter treatments were less effective. The rate of secretion of oestriadiol from the ovary on days 2–5 of pregnancy in control rats was low in the morning and high in the afternoon. Treatment with 4-OH-A from days 2 to 5 reduced the secretory surges of oestradiol in the afternoon by 77% without significantly changing the progesterone output. Systemic testosterone levels were significantly increased by this treatment.

To assess whether changes in the transport of ova were due to an increase in testosterone concentrations the influence of exogenous testosterone on embryo transport and oestradiol production was tested. Testosterone administered by subdermal implants from days 2 or 3 to day 5 disturbed embryo transport in a manner similar to that of 4-OH-A. The longest period of testosterone administration decreased ovarian oestradiol production by 82% without changing the secretion of progesterone.

Since ovarian oestradiol production in pregnant rats was reduced similarly by treatment with 4-OH-A and testosterone implants, and since exogenous oestradiol given s.c. on days 2, 3 and 4 counteracted the blocking effect of 4-OH-A and testosterone on embryo transport, the delay in oviducal transport of the embryos can be accounted for by decreased availability of oestradiol rather than increased testosterone production. This interpretation supports a crucial role for endogenous oestradiol in timing the passage of embryos to the uterus in the pregnant rat.

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INTRODUCTION

The physiological role of ovarian steroids in the regulation of oviducal transport of ova has been inferred mostly from alterations in ovum transport which result when animals are given exogenous hormones in excess (for review see Harper, Pauerstein, Adams et al. 1976). The finding of differences in ovum transport between pregnant and cyclic rats, associated with different endogenous steroid levels (Forcelledo, Vera & Croxatto, 1981), provides a model with which to obtain further evidence in support of a physiological involvement of ovarian steroids in the regulation of the rate of egg transport.

The number and distribution of ova between oviducts and uterus is quite similar in cyclic and pregnant rats during the first 3 days after ovulation, but significant differences appear thereafter. The major shift in the location of ova takes place between days 3 and 4 of the oestrous cycle (dioestrus and pro-oestrous respectively) and between days 4 and 5 of pregnancy. In cyclic rats about 60% of ova disappear from the genital tract on day 4, contrasting with the situation in pregnant rats in which the majority of embryos remain in the oviducts. Cyclic animals exhibit earlier transfer of ova to the uterus and partial failure to retain them in comparison with pregnant rats (Forcelledo et al. 1981). These temporal differences


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in the pattern of ovum transport may be due to the significantly lower oestrogenic secretion observed during the period of egg transport in pregnant animals as compared with values observed in cyclic animals (Shaikh, 1971). On the other hand, circulating progesterone levels increase similarly in cyclic and pregnant rats during the first 3 days after ovulation but, while they return to basal levels on day 4 in cyclic rats, they continue to rise up to day 5 in pregnant rats (Forcelledo et al. 1981). It thus appears that the faster transport of ova in the oestrous cycle, compared with that in pregnancy, is associated with a relative oestrogenic predominance in the former situation. The experimental demonstration of oviducal retention of ova associated with reduced oestadiol secretion during the oestrous cycle supports such an interpretation (Forcelledo & Croxatto, 1986).

Further support along this line was sought in the pregnant rat using the aromatase inhibitor 4-hydroxyandrostenedione (4-OH-A) to decrease oestrogen secretion. Since this treatment increases testosterone levels at the same time as it lowers oestriadiol secretion (Brodie, Schwarzel, Shaikh & Brodie, 1977), a number of control experiments are required to provide evidence that the effects on embryo transport are due solely to reduced availability of oestrogen. These include substitution therapy with oestradiol, which should reverse the effects of 4-OH-A, and administration of testosterone, which should not mimic the effects of 4-OH-A on egg transport.

**MATERIALS AND METHODS**

**Animals**

Female Sprague–Dawley rats (Charles River, Wilmington, MA, U.S.A.) weighing 220–250 g were used. They were kept with lights on from 07.00 to 21.00 h at a room temperature of about 25 °C, and caged on pro-oestrus with males of proven fertility. Mating was verified by observation of spermatozoa in the vaginal smear on the following morning; this was designated day 1 of pregnancy.

The aromatase inhibitor 4-OH-A (supplied by Dr A. Brodie, University of Maryland, Baltimore, MD, U.S.A.) was injected s.c. in 0·1 ml propylene glycol: dimethylsulphoxide (1:1, v/v) every 4 h for the periods indicated below. Each injection contained 1·66 mg inhibitor so that the total dose/24 h per rat was 10 mg. Control animals received the same volume of vehicle at the same times. Some animals were killed at the end of the treatment to determine the number and distribution of embryos. Other animals were used to determine ovarian oestriadiol and progesterone secretion as well as systemic testosterone levels at different times after the onset of treatment.

**Embryo transport**

To assess the occurrence of a delay in oviducal embryo transport, rats were killed at about midday on day 5 by ether inhalation. Oviducts and uterine horns were removed and flushed separately with physiological saline. All flushings were examined under low-power magnification to count the embryos.

**Collection of ovarian and systemic blood**

The genital tract was exposed under light ether anaesthesia through a ventral mid-line incision, and heparin was injected through the femoral vein. The uterine vein was ligated and polyethylene tubing, fitted with a stainless-steel needle point (gauge 26), was inserted into the ipsilateral ovarian vein. Blood was collected into a centrifuge tube for 6–20 min. The time of collection, blood volume and haematocrit were recorded. After collection of ovarian blood, systemic blood was obtained by aortic puncture. All plasma samples were kept at −20 °C until radioimmunoassay.

**Hormonal determinations**

Radioimmunoassays were carried out according to the procedures of the WHO Programme for the Provision of Matched Assay Reagents for the RIA of Hormones in Reproductive Physiology.

Plasma oestriadiol was assayed utilizing an antisera (provided by Dr E. D. B. Johansson, Uppsala, Sweden) raised in rabbits against oestriadiol-6-O-carboxymethylxime conjugated with bovine serum albumin (BSA). Plasma (100 μl) was extracted twice with 1 ml diethylether after adding a tracer amount of tritiated oestriadiol to correct for procedural losses. Recovery fluctuated between 85 and 95%. The antisera was used at a final dilution of 1:200 000 and the lower limit of sensitivity was 5·5 pmol/tube. Serial dilutions of plasma samples gave slopes parallel with the oestriadiol standard curve. Water blank values were indistinguishable from zero. Coefficients of variation between assays were 23 and 17·9% for the low and high pool respectively. The corresponding intra-assay variations were 9·4 and 7·3%. Cross-reaction with oestrone at 50% binding was 16·2%.

Progesterone concentrations were measured using an antisera raised in rabbits against progesterone-3-carboxymethylxime conjugated with BSA. Plasma (1:100 dilution; 20 μl) was extracted with 1·5 ml diethylether after adding a tracer amount of [3H]progesterone to correct for procedural losses. Recovery fluctuated between 80 and 90%. The antisera was used at a final dilution of 1:56 000 and the lower limit of sensitivity was 15·9 pmol/tube. Water and ether values were indistinguishable from zero. Coefficients of variation between assays were 31·3 and 17·0% for the low and high pool respectively. The
corresponding intra-assay variations were 20.5 and 10.2%. This antiserum cross-reacted 2.7% with 20a-dihydroprogesterone and less than 1% with testosterone at 50% binding.

Plasma testosterone was assayed utilizing a monoclonal antiserum raised in mice against testosterone-3-carboxymethylxime conjugated with BSA. Plasma (50 μl) was extracted with 1.5 ml diethylether after adding a tracer amount of $[^3]$H]testosterone to correct for procedural losses. Recovery was 93%. The antiserum was used at a final dilution of 1:210,000 and the sensitivity was 5-9 pmol/tube. Water and ether blank values were indistinguishable from zero. All samples were measured in the same radioimmunoassay and the intra-assay variations were 12-5 and 5% for the low and high pool respectively. Cross-reaction with 4-androstenedione at 50% binding was 3.5% and less than 0.002% with 4-OH-A.

Progesterone and testosterone antisera and reagents for the radioimmunoassays were provided by the WHO Programme for the Provision of Matched Assay Reagents for the RIA of Hormones in Reproductive Physiology (WHO Collaborating Centre, Chelsea Hospital for Women, London).

Experiments

The following experiments were performed using the methods described above.

Experiment 1

This experiment was performed to assess the influence of 4-OH-A on embryo transport. Rats were treated with 4-OH-A from 12.00 h on days 2, 3 or 4 of pregnancy to 08.00 h on day 5 (ten, thirteen and four pregnant rats respectively). These modes of administration will be referred to as the 2–5 days, 3–5 days and 4–5 days of 4-OH-A treatment. Control rats received vehicle only (five, six and three rats respectively) under the same schedule. All rats were killed at about midday on day 5.

Experiment 2

The purpose of this experiment was to assess the effects of 4-OH-A on ovarian production of oestradiol and progesterone and on the systemic levels of progesterone. In eight rats given 2–5 days of 4-OH-A treatment (see experiment 1) ovarian venous blood and peripheral blood were collected at 16.00 h on days 2 and 4 of pregnancy (n = four rats at each sampling time). Due to the limited availability of 4-OH-A it was not possible to treat as many rats as required to match the sampling schedule of the control group. Pregnant rats injected with the vehicle, using the same schedule as that for rats treated with 4-OH-A for 2–5 days, were sampled to assess the basal ovarian secretion of oestradiol or progesterone. Ovarian venous blood was collected at 16.00 h (n = four) and 20.00 h (n = four) on day 2; at 12.00 h (n = three), 16.00 h (n = three) and 20.00 h (n = six) on day 3; at 08.00 h (n = four), 12.00 h (n = four), 16.00 h (n = five) and 20.00 h (n = four) on day 4 and at 12.00 h (n = four) and 16.00 h (n = four) on day 5. Peripheral blood was collected at 16.00 h on days 2, 3 and 4 of pregnancy in other rats to assess the systemic concentrations of testosterone (n = four, five and four respectively).

Experiment 3

This experiment was designed to assess the influence of increased testosterone levels on the transport of embryos. Pregnant rats were implanted s.c. with two Silastic (Dow Corning Corporion, Midland, MI, U.S.A.) capsules (34 mm long, 1.57 mm inner diameter and 3.17 mm outer diameter). Each capsule was filled with 25.6 ± 1.5 mg testosterone crystals (Sigma Chemical Co., St Louis, MO, U.S.A.) and sealed with adhesive (2 mm) at each end. Testosterone implants were incubated in saline at 37°C for 24 h before use. They were implanted at 12.00 h on day 2 or 3 (eight and seven rats respectively) and left until autopsy at midday on day 5. Control animals received empty capsules (eight rats each).

Experiment 4

This experiment was performed to assess the effect of testosterone implants on ovarian secretion of oestradiol and progesterone and on the systemic concentrations of testosterone. Twelve rats were implanted at 12.00 h on day 2 of pregnancy with Silastic capsules containing testosterone crystals (see experiments 2 and 3). Ovarian venous blood and systemic blood were collected at 16.00 h on days 3 and 4 of pregnancy (six rats each).

Experiment 5

In this experiment we tested the effects of replacement therapy with oestradiol on the transport of embryos in rats treated with either 4-OH-A or testosterone. Rats were treated for 2–5 days with 4-OH-A (see experiment 1) or with testosterone from 12.00 h on days 2–5 of pregnancy (six and five rats respectively). These animals were injected s.c. at 17.00 h on days 2, 3 and 4 of pregnancy with 0.25 μg oestradiol (Sigma Chemical Co.) dissolved in 0.1 ml propylene glycol. Animals treated on days 2–5 with 4-OH-A (ten rats) or testosterone (eight rats) were the same as those in experiments 1 and 3 respectively. Another six rats were injected s.c. with 0.25 μg oestradiol only, on days 2, 3 and 4 of pregnancy to assess whether this dose of oestradiol would accelerate embryo transport in animals with normal endogenous production of oestrogen. Five control rats were injected with vehicle and implanted with empty Silastic capsules. All rats were killed at about midday on day 5.
Statistical analysis

Analysis of variance was used to compare the distribution and recovery of embryos in treated and control groups (Conover & Iman, 1981). Differences in ovarian secretion of oestriadiol and progesterone, and differences in plasma concentrations of testosterone between treated and control groups were assessed by Student's t-test after logarithmic transformation of data. Only P values <0.05 were considered statistically significant.

RESULTS

Experiment 1

The number and distribution of embryos recovered from the genital tract on day 5 of pregnancy in control rats and in those treated with 4-OH-A given from days 2, 3 or 4 to day 5 are shown in Fig. 1. All the embryos were recovered from the uterus in control rats and no statistically significant differences were observed in the number and distribution of embryos between the three control groups. No differences were found in the total number of embryos recovered between control groups and rats injected with the inhibitor. The distribution of embryos differed significantly between groups on 2-5 days and 3-5 days of 4-OH-A treatment and the controls. In contrast with control animals in which all the eggs had been transported to the uterus, 47 and 21% of the embryos were retained in the oviducts in the groups on 2-5 days and 3-5 days of 4-OH-A treatment respectively. These differences in distribution with the control group were statistically significant (P<0.01 and P<0.05 respectively). No effect was observed in the groups on 4-5 days of 4-OH-A treatment.

Experiment 2

Ovarian oestradiol secretion during the first 5 days of pregnancy in control rats and in rats under 2-5 days of 4-OH-A treatment is shown in Fig. 2. In control rats, higher secretory rates of oestradiol were found during the afternoon on days 2, 3 and 5 of pregnancy in comparison with values observed in the morning. The increase in oestradiol secretion that occurred during the afternoon on day 4 did not differ significantly from values obtained in the morning. Oestradiol secretion was reduced (77% average) below the respective control levels by administration of 4-OH-A. Values for secretion at 16.00 h on days 2 and 4 were significantly (P<0.02) lower in treated than in control animals.

Table 1 shows the effect of 2-5 days of 4-OH-A treatment on the ovarian secretion of progesterone, the systemic testosterone levels and the rate of ovarian blood flow. Progesterone secretion increased from days 2 to 4 without a significant difference between control rats and those treated with 4-OH-A. Systemic testosterone levels increased significantly from days 2 to 4 in treated rats and were 5- to 24-times above their respective controls. There were no significant differences in the ovarian blood flow between control rats and those treated with 4-OH-A.

Experiment 3

The effect of testosterone administration on embryo transport on day 5 of pregnancy is shown in Fig. 3. All embryos were recovered from the uterus in both control groups. No differences were found in the total number of embryos between control and testosterone-treated rats. Testosterone administered from days 2 or 3 to day 5 produced oviducal retention of 47.8% (P<0.0002) and 12.6% (non-significant) of the embryos respectively.

Experiment 4

Since treatment with testosterone had the same effect as 4-OH-A on embryo transport, its effect on oestradiol secretion was also studied. The ovarian secretion of oestradiol and progesterone, the systemic concentration of testosterone and the rate of blood flow in

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agreement with the acceleration of transport produced by this treatment in the rat. However, only 0.25 µg oestradiol given alone in the afternoon on days 2, 3 and 4 decreased significantly \( (P<0.005) \) the total number of embryos recovered from uteri on day 5 compared with that in control rats. In addition, there were significant \( (P<0.0001) \) differences in the distribution of embryos between rats treated with 4-OH-A or testosterone alone and those receiving oestradiol in addition to 4-OH-A or testosterone. No eggs were retained in the oviducts of rats treated concomitantly with oestradiol and 4-OH-A or testosterone.

DISCUSSION

The present experiments show that 4-OH-A and testosterone delay embryo transfer from oviduct to uterus if given from days 2 or 3 to day 5 of pregnancy. Testosterone implants and 4-OH-A administered from day 2 caused retention of nearly 50% of the eggs in the oviducts at midday on day 5. A smaller proportion of embryos was retained with hormonal treatments beginning on day 3. Testosterone and 4-OH-A significantly reduced ovarian oestriadiol secretion and increased systemic testosterone levels without parallel changes in the ovarian progesterone output. The associated effects on egg transport were completely reversed by concomitant treatment with oestradiol.

Systematic measurements of ovarian oestriadiol secretion on days 2–5 of pregnancy disclosed increments during the afternoon. To the best of our knowledge this is the first description of rhythmic ovarian oestriadiol secretion during the oviducal transport of embryos. Apparently, day 4 was the only one previously screened in sufficient detail to show this rhythm (Shaikh, 1971).

Treatments with 4-OH-A or testosterone caused an average 80% reduction in ovarian oestriadiol secretion measured during the afternoon. While it is well established that 4-OH-A inhibits oestriadiol synthesis \( \textit{in vitro} \) (Brodie, Garret, Hendrickson \textit{et al.} 1981), the reduction seen in ovarian output of oestriadiol \( \textit{in vivo} \) could result solely from decreased blood flow through the ovary. Clearly this was not the case, at least on day 3. In addition, the increase in testosterone levels in the circulation is in agreement with a reduction of the conversion of androgen to oestrogen. Inhibition of the oestrogen synthetase system therefore offers a common explanation for the observed hormonal changes following treatment with 4-OH-A.

The reduction of ovarian oestriadiol output on days 3 and 4 of pregnancy caused by sustained administration of testosterone has not, to our knowledge, been described previously. It could be explained by reduced blood flow through the ovary, by inhibition

FIGURE 2. Inhibition of ovarian oestradiol secretion by 4-hydroxyandrostenedione (4-OH-A) in pregnant rats. Animals were injected (arrows) s.c. with vehicle (○) or 1.6 mg 4-OH-A (●) every 4 h from 12.00 h on day 2 until collection of ovarian venous blood. The horizontal bar with open and shaded areas shows light and dark periods respectively. Three to six rats were used at each time. \( *P<0.02 \) compared with control (Student’s \( t \)-test).

Experiment 5

Table 3 shows the effect of oestradiol given alone and in conjunction with 4-OH-A or testosterone on the transport of embryos. In all oestradiol-treated groups the absolute number of embryos was decreased in rats with or without testosterone implants are shown in Table 2. Ovarian oestradiol production at 16.00 h on days 3 and 4 was significantly decreased in treated animals to 80% \( (P<0.01) \) and 86% \( (P<0.003) \) respectively of the values in the controls. Progesterone output was slightly but not significantly decreased, whereas systemic testosterone levels were significantly \( (P<0.0001) \) increased, being 16 times above their respective controls at all times. The ovarian blood flow was decreased on day 4 in testosterone-treated rats \( (P<0.05) \).
of gonadotrophin secretion or by a mechanism similar to that of 4-OH-A. Based on indirect evidence it was previously considered that testosterone had no inhibitory effect on aromatase (Brodie et al. 1977). Further experiments will have to be performed to resolve this question.

The oviducal effects of 4-OH-A or testosterone could be related to a deficit of oestradiol and/or an excess of testosterone. Since oestradiol replacement therapy was able to revert completely the oviducal effects of treatment with 4-OH-A or testosterone, we believe that the delay in the oviducal transport of embryos is due to the deficit of oestrogens rather than to the excess of androgens. The sustained progesterone secretion concomitant with a lower oestrogen production decreased the oestradiol/progesterone ratio in blood. Such a change, or the absolute decrease of oestradiol, could be responsible for the slower transport.

The disturbance of embryo transport produced by 4-OH-A or testosterone was more pronounced the earlier the onset of treatment. This suggests that the hormonal signals which time the passage of embryos to the uterus are active as early as day 2 of pregnancy. Administration of 4-OH-A from day 4 did not change the time of passage of eggs to the uterus, indicating that the ovarian oestradiol surge on day 4 is not essential for this event.

Other androgenic compounds, such as androstenedione (4 or 8 mg/day) or dehydroepiandrosterone (2.5, 5 or 10 mg/day), given to rats from days 2 to 5 caused the expulsion of eggs from the reproductive tract before implantation (Harper, 1967, 1969). Both androgens are known to be aromatized to oestrogens in vitro (Schwarzel, Krugel & Brodie, 1973) and the increased oestrogen production may be responsible for the accelerated transport of eggs. At the present time we have no explanation for the different response to testosterone as compared to androstenedione or dehydroepiandrosterone.

In contrast to the findings in pregnant rats, a previous study in cyclic rats (Forcelledo & Croxatto, 1986) showed that treatment with testosterone did not produce a significant retention of ova in the oviducts. Treatment with 4-OH-A during late metoestrus and dioestrus, however, had the same qualitative effect as in pregnant rats; this effect was also reversed by concomitant oestradiol administration. Because the cyclic rat has a higher oestradiol production than the pregnant animal at comparable times after ovulation, it

TABLE 1. Ovarian secretion of progesterone, systemic concentration of testosterone and rate of ovarian blood flow in control and 4-hydroxyandrostenedione (4-OH-A)-treated pregnant rats. Values are means ± S.E.M.; numbers of rats are given in parentheses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day of pregnancy</th>
<th>Progesterone (µmol/h per ovary)</th>
<th>Testosterone (nmol/l)</th>
<th>Blood flow rate (ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>2</td>
<td>5.5 ± 3.8 (4)</td>
<td>0.32 ± 0.04 (4)</td>
<td>3.5 ± 1.9 (4)</td>
</tr>
<tr>
<td>4-OH-A</td>
<td>2</td>
<td>3.7 ± 0.5 (4)</td>
<td>1.66 ± 0.34 (4)*</td>
<td>3.0 ± 0.4 (4)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>4</td>
<td>11.8 ± 0.3 (3)</td>
<td>0.31 ± 0.02 (4)</td>
<td>5.3 ± 0.9 (5)</td>
</tr>
<tr>
<td>4-OH-A</td>
<td>4</td>
<td>10.0 ± 2.3 (4)</td>
<td>7.56 ± 1.97 (4)*</td>
<td>3.8 ± 0.7 (4)</td>
</tr>
</tbody>
</table>

*P < 0.0001 compared with respective control (Student's t-test).
Rats were given 1.66 mg 4-OH-A or vehicle every 4 h from 12.00 h on day 2 until blood collection at 16.00 h on days 2 and 4 as described in Materials and Methods.

FIGURE 3. Effect of testosterone (stippled bars) on the distribution of rat embryos on day 5 of pregnancy. Testosterone capsules were implanted s.c. at 12.00 h on day 2 or 3 and left until autopsy (day 5). Values are means ± S.E.M.; numbers of animals are given in parentheses. *P < 0.0002 compared with control rats (open bars; analysis of variance).

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**TABLE 2.** Ovarian secretion of oestradiol and progesterone, systemic concentration of testosterone and rate of ovarian blood flow in control and testosterone-treated pregnant rats. Values are means ± s.e.m.; numbers of rats are given in parentheses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oestradiol (nmol/h per ovary)</th>
<th>Progesterone (nmol/h per ovary)</th>
<th>Testosterone (nmol/l)</th>
<th>Blood flow rate (ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>2.35 ± 1.25 (3)</td>
<td>3.9 ± 1.5 (6)</td>
<td>0.27 ± 0.06 (5)</td>
<td>2.9 ± 0.5 (3)</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.45 ± 0.10 (6)**</td>
<td>4.26 ± 0.41 (6)****</td>
<td>4.99 ± 0.45 (6)****</td>
<td>2.6 ± 1.0 (6)*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>4.81 ± 1.64 (5)</td>
<td>11.8 ± 0.3 (3)</td>
<td>0.31 ± 0.02 (4)</td>
<td>3.0 ± 0.9 (5)</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.68 ± 0.17 (6)***</td>
<td>8.2 ± 2.6 (6)</td>
<td>8.2 ± 1.4 (6)</td>
<td>8.2 ± 1.4 (6)</td>
</tr>
</tbody>
</table>

*p < 0.05, **P < 0.01, ***P < 0.003, ****P < 0.0001 compared with respective control (Student's *t*-test).

Testosterone-treated rats were implanted at 12.00 h on day 2 with Silastic capsules containing testosterone, and ovarian vein and systemic blood was collected at 16.00 h on days 3 or 4. Controls are the same as those of experiment 2 (see Materials and Methods).

**TABLE 3.** Effects of oestradiol on embryo transport in pregnant rats treated with 4-hydroxyandrostenedione (4-OH-A) or testosterone. Values are means ± s.e.m.; numbers of rats are given in parentheses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oviduct</th>
<th>Uterus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0 ± 0.0</td>
<td>6.9 ± 1.6</td>
<td>6.9 ± 1.2 (6)*</td>
</tr>
<tr>
<td>Oestradiol</td>
<td></td>
<td>5.9 ± 1.8</td>
<td>11.1 ± 1.4 (10)</td>
</tr>
<tr>
<td>4-OH-A</td>
<td></td>
<td>8.2 ± 1.4†</td>
<td>8.2 ± 1.4 (6)</td>
</tr>
<tr>
<td>4-OH-A + oestradiol</td>
<td></td>
<td>6.0 ± 1.4</td>
<td>11.5 ± 0.8 (8)</td>
</tr>
<tr>
<td>Testosterone</td>
<td>9.2 ± 1.5†</td>
<td>9.2 ± 1.5 (5)</td>
<td></td>
</tr>
<tr>
<td>Testosterone + oestradiol</td>
<td>9.2 ± 1.5†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05 compared with control; 1P < 0.0001 compared with 4-OH-A or testosterone alone (analysis of variance).

Control rats were injected with vehicle and implanted with empty Silastic capsules. Oestradiol, 0.25 µg injected s.c. at 12.00 h on days 2, 3 and 4; 4-OH-A, 166 mg injected s.c. every 4 h from 12.00 h on day 2 to 08.00 h on day 5; Testosterone, two Silastic capsules implanted s.c. at 12.00 h on day 2 and left until autopsy. All rats were killed at about midday on day 5.

is likely that treatment with testosterone in the former condition was less effective in decreasing oestradiol levels. Unfortunately, this was not investigated because testosterone had no effect on the transport of the ova.

In summary, oestradiol secretion was decreased by the administration of 4-OH-A, a competitive inhibitor of oestrogen synthetase, and by an excess of testosterone. Both treatments produced an increase in circulating testosterone levels and oviducal retention of embryos. The latter effect was completely reversed by exogenous oestradiol. It was thus confirmed that a pronounced decrease in basal oestradiol secretion would delay the entry of the embryos to the uterus. This taken together with the fact that an excess of oestradiol alone accelerates the rate of embryo transport (Ortiz, Villalón & Croxatto, 1979) indicates that the level of endogenous oestradiol secretion up to day 3 of pregnancy is crucial in timing the transfer of eggs from the oviduct to the uterus in cyclic and pregnant rats.

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